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DEVELOPMENT OF A LEGUME INOCULANT PROGRAMME IN JAMAICA

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ABSTRACT

In Jamaican soils, the indigenous population of rhizobia are low and are generally ineffective. This usually results in poor nodulation of important grain legumes such as red pea (Phaseolus vulgaris) and cowpea (Vigna unguiculata), thus depriving the plant of needed nitrogen. A legume inoculant project has been initiated by the Scientific Research Council (SRC) which is aimed at producing good quality peat based inoculants to improve the yields of red pea and other legume crops. Rhizobium phaseoli strains obtained from CIAT and indigenous isolates were screened for their nitrogen fixing ability on red pea. The ability of selected rhizobia to survive in peat and to form effective nodules on the host plant under sterile conditions after prolonged storage at two temperatures were also examined. Results indicate that R. phaseoli strains obtained from CIAT were better suited than indigenous isolates for uses as inoculants. Inoculants had $>10^7$ rhizobia per g peat up to six (6) months storage at $30^{o}C$ and $8^{o}C$ and were capable of forming effective nodules on red pea under sterile conditions.

-- KEY WORDS: Inoculant; Rhizobium sp; Nitrogen fixation

INTRODUCTION

It has been known for many years that the legume - Rhizobium symbiotic association can provide a highly economical source of available nitrogen for legume crops (Erdman, 1959). In tropical soils, the benefits of this association are not being fully utilized. In Jamaican soils, the indigenous populations of rhizobia are low and are generally ineffective in their ability to fix nitrogen (Ahmad and McLaughlin, 1985; Uddin et al, 1981). Low and ineffective populations of rhizobia usually result in poor nodulation of important grain legumes such as red pea (Phaseolus vulgaris) and cowpea (Vigna unguiculata).

Although red pea and cowpea have been grown in Jamaica for many years, little or no attention has been given to nodulation and nitrogen fixation. Legume crops are generally grown by small farmers in Jamaica without the use of legume inoculants and low inputs of chemical nitrogen fertilizer. Yields of red pea and cowpea obtained are generally low, with average yields of 800 lbs/acre and 860 lbs/acre respectively (Data Bank, Ministry of Agriculture). To supplement local demands, it is estimated that more than 2 million pounds of red pea are imported into Jamaica annually.

The improvement of legume yields and the establishment of effectively nodulated legumes can be achieved by using legume inoculants which contain effective and competitive strains of rhizobia. However, the supply of

quality legume inoculant is limited mostly to developed countries. Also, developing countries generally lack a regular supply of quality legume inoculants.

Inoculants are prepared by mixing rhizobia cultures with carrier materials. The most widely used carrier is dried, milled peat. Although peat is the best carrier identified to date, it is not readily available in the tropics and its suitability varies depending on the source.

This work was undertaken to identify suitable rhizobial strains, in particular R. phaseoli which could be used to prepare inoculants for red pea. Secondly, to evaluate peat mined from different areas from the western coastal regions of Jamaica for their general suitability as a carrier for inoculant production.

MATERIALS AND METHODS

Rhizobia

Rhizobium strains were either isolated from nodules locally (Uddin et al, 1981) or obtained from culture collections. R. phaseoli strains 144, 643, 640 and 652 were obtained from Centro Internationl de Agricultura Tropical (CIAT). Cultures were maintained on yeast extract mannitol (YEM) agar slopes, pH 6.8, prepared essentially as described by Ahmad et al, 1981.

Peat

Peat used in this study was mined in Black River, Negril and Sheffield. The peat was air dried and then ground in a hammer mill and sifted to pass a 100-mesh sieve. The pH and moisture holding capacity were determined as described by Somasegaran and Hoben, 1985. The pH of the peat was adjusted to 6.5 using precipitated $CaCO_3$ (BDH). The ground peat was autoclaved for three consecutive days each for one hour. A 20 g peat sample was then packaged aseptically in sterile 5" x 8" polyethylene bags.

Preparation of Inoculant

R. phaseoli strains were grown in 500 ml culture flasks containing 200 ml YEM broth in an incubator shaker for 72 hours at 30°C (O.D. = 1.0). Broth cultures (10 ml) were added to each bag of (20 g) peat and the bags heat sealed. Each bag was kneaded thoroughly to ensure proper mixing of the broth culture with the peat. One set of these packages was incubated at 30°C and the other set at 8°C for up to 180 days. Controls were prepared by inoculating peat with sterile YEM broth and incubated at the same temperatures.

Enumeration of Rhizobia

The number of viable rhizobia in the inoculant was determined at regular intervals by plate count. A 10 g amount of inoculant was aseptically transferred to 90 ml of saline (0.85% NaCl) and shaken for 2 mins. After mixing, the suspension was serially diluted in saline and

plated, in duplicates, on YEM agar plates containing congo red (Vincent, 1970). Colonies were counted after the plates had been incubated for 72 hours at 30°C.

Plant Infection Test

Phaseolus vulgaris (var Portland red) seeds were surfaced sterilized by washing for 2 mins in 0.1% (w/v) HgCl, and rinsed ten times with distilled water. The seeds were then germinated and seedlings planted in 350 ml wide mouth polypropylene jars containing sterile vermiculite and 0.5 g inoculant added to each jar.

At planting, 12 mg nitrogen per jar was applied in the form of ammonium sulphate, after which N-free solution, alternated with sterile distilled water, was added. At 35 days after planting, the plants were harvested, and the roots of each plant were examined for the presence of nodules. Total shoot nitrogen was determined (Kjeldahl).

RESULTS AND DISCUSSION

Rhizobium phaseoli strains obtained from Centro International de Agricultura Tropical (CIAT) and indigenous isolates were screened for their nitrogen fixing ability on Phaseolus vulgaris var Portland red (red pea). In general, it was found that indigenous strains were ineffective whereas CIAT strains formed effective nodules on the host plant. As a result CIAT strains were selected for further studies.

The ability of rhizobia to survive, multiply and maintain effectiveness in a particular peat must be examined before the peat is selected for inoculant production (Somasegaran and Hoben, 1985). Peat used in this study was mined in Black River, Negril and Sheffield (western coastal region of Jamaica). Analysis of the peat used showed that the pH ranged from 5.4 to 6.8, and the moisture holding capacity 90 to 94 ml/100 g.

Tables 1, 2 and 3 show the survival of four strains of R. phaseoli in inoculants prepared with each of the three peat. All peat examined supported the growth and survival of all strains of R. phaseoli except for peat mined from Sheffield (Table 1). R. phaseoli 640 decreased to less than 10^3/g of peat in less than 15 days in Sheffield peat.

The influence of storage temperature on survival of R. phaseoli strains was studied by comparing viable counts of inoculants stored at 30°C and 8°C. The effect of storage temperatures had no significant effect ($P = 0.05$) on the survival of the Rhizobium strains (except for 640, Table 1). In most cases there was an improvement in the numbers of rhizobia. Low storage temperatures for inoculants have been reported to reduce dessication and thus have a favourable effect of rhizobial survival (Roughley, 1968; Aarons and Ahmad, 1986). In this work, it was found that low temperature storage was not essential for the survival of inoculants. Storage at room temperature would be quite advantageous since most small farmers will probably not own a refrigerator in which inoculant would normally be stored.

After 60 days storage, peat inoculants were used in plant infection tests (Vincent 1970) to examine the ability of the inoculants to nodulate

red pea (Table 4). All inoculants tested nodulated the host plant and all were effective as tested by the total shoot nitrogen content. Statistical analysis (Duncan's multiple range test at P = 0.05) indicated that the storage temperature did not affect the effectiveness of the inoculants.

Our results indicate that peat located in the western coastal region appears to be suitable as a carrier in the preparation of inoculants because the numbers of rhizobia recovered from peat inoculants after long term storage were acceptable to given standards (Date and Roughley, 1977). Similar results were observed when the survival of slow-growing cowpea rhizobia were examined in peat obtained from Black River (Aarons and Ahmad, 1986). Results also indicate that R. phaseoli CIAT strains could be used for the preparation of inoculants.

Due to continuous farming without nitrogen replenishment, low indigenous rhizobial populations (cowpea rhizobia 2.8×10^2 cells/g soil; R. phaseoli <10/g soil) support the idea that the response of legumes to inoculation will be positive. Inoculation is not expensive and the cost benefit ratio is potentially very high. Inoculation is, therefore, economically justified in many legume crops. Farmers can buy inexpensive inoculants as compared to costly nitrogen fertilizers to improve grain legume yields.

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Table 1 Survival of *R. phaseoli* strains in peat mined from Sheffield, Westmoreland

R. Phaseoli Inoculant	Storage Condition	Log_{10} colony-forming units per g after:						
		0d	15d	30d	45d	60d	75d	90d
CIAT 144	30°C	8,11	8,65	ND	8,89	9,23	9,68	8,50
CIAT 144	8°C	8,11	9,04	ND	8,77	8,30	9,93	8,78
CIAT 632	30°C	10,64	ND	9,99	9,49	7,81	5,70	5,60
CIAT 632	8°C	10,64	10,67	10,63	9,80	7,40	6,87	6,80
CIAT 640	30°C	8,87	<3,00	ND	NO	ND	ND	ND
CIAT 640	8°C	8,87	7,18	4,23	<3,00	ND	ND	ND
CIAT 652	30°C	8,54	8,84	NO	9,86	8,32	9,48	9,30
CIAT 652	8°C	8,54	9,18	ND	8,40	8,16	9,80	9,28

Table 2 Survival of *R. phaseoli* strains in peat mined from Negril (PCJ) Westmoreland

R. phaseoli Inoculant	Storage Condition	Log_{10} colony-forming units per g after:						
		0d	30d	45d	60d	75d	90d	180d
CIAT 144	30°C	9.23	10.42	10.74	11.50	10.29	10.86	10.02
CIAT 144	8°C	9.23	10.42	10.74	11.50	10.29	10.86	10.02
CIAT 632	30°C	9.39	11.03	11.12	11.27	11.48	11.83	10.03
CIAT 632	8°C	9.39	10.95	10.72	10.33	10.41	9.74	11.78
CIAT 640	30°C	9.48	10.60	11.27	10.20	11.70	10.54	9.60
CIAT 640	8°C	9.48	11.00	10.88	10.59	10.47	10.33	9.43
CIAT 652	30°C	9.13	11.36	11.58	11.57	11.98	11.82	10.28
CIAT 652	8°C	9.13	11.28	11.54	11.60	11.89	11.93	10.61

Table 3 Survival of *R. phaseoli* strains in peat mined from Black River, St. Elizabeth

R. phaseoli Inoculant	Storage Condition	Log_{10} colony-forming unit per g after:						
		0d	30d	45d	60d	75d	90d	180d
CIAT 144	30 C	10.02	11.67	11.28	11.16	11.38	10.22	9.65
CIAT 144	8 C	10.02	11.58	11.33	11.36	11.37	10.08	9.77
CIAT 632	30 C	10.31	11.22	11.63	11.12	12.98	11.80	9.88
CIAT 632	8 C	10.31	11.31	11.75	11.19	12.12	12.30	9.95
CIAT 640	30 C	10.23	11.82	11.71	11.07	12.48	10.21	9.10
CIAT 640	8 C	10.23	11.38	11.59	11.17	12.49	10.54	9.15
CIAT 652	30 C	9.77	11.28	11.92	11.45	12.28	10.70	9.45
CIAT 652	8 C	9.77	11.51	11.86	11.42	12.55	10.35	9.96

Table 4 Effectiveness of *R. phaseoli* Peat-based inoculant (Sheffield Peat) after 60 days storage on red pea (*P. vulgaris*) car Portland red).

R. phaseoli Inoculant	Storage Condition	Nodule no.	Nodule dry wt. (mg)	Shoot dry wt. (g)	Total Shoot N (% N)
CIAT 144	30°C	383.50[a]	142.78[a]	1.24[a]	2.60[a]
CIAT 144	8°C	320.75[a]	173.00[a]	1.21[a]	2.57[a]
CIAT 632	30°C	334.00[a]	150.37[a]	1.09[a]	3.06[a]
CIAT 632	8°C	227.25[b]	131.87[a]	1.17[a]	2.37[a]
CIAT 652	30°C	252.50[b]	119.50[a]	1.14[a]	3.49[b]
CIAT 652	8°C	278.25[b]	107.25[a]	0.83[a]	2.58[a]
Control	30°C	0.0	0.0	0.53[b]	1.71[c]
Control	8°C	0.0	0.0	0.58[b]	1.62[c]

Statistical comparisons: numbers followed by similar letters (a, b, or c) are significantly different at P = 0.05 by Duncan's multiple range test.